"Evaluation of Isoflavonoid Genistein induced antioxidant activity in human breast cancer cell lines".

SYNOPSIS

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1. "Evaluation of Isoflavonoid Genistein induced antioxidant activity in human breast cancer cell lines".

Free radicals are an atom, group, or molecule, which contains one or more unpaired electrons (Euis R.Y., 2018). The biochemical reactions of respiration and metabolism in the reactive oxygen species (ROS) or free radicals are formed (Cui H et al., 2012). ROS plays a role in physiological processes, such as reducing inflammation, killing bacteria, and regulating blood vessel and organ smooth muscle tone (Euis R.Y., 2018). However, the excessive increase in ROS production in the body can reduce the number of cells, tissues, and the incidence of various diseases (Sen B.D.S., et al., 2010). In the neutralization of ROS, the body naturally has defences, namely endogenous antioxidants such as the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (Sanmugapriya E and Venkataraman S., 2006). Antioxidants work to avoid the damage of cells and tissues, thereby inhibiting the body's oxidative reactions (Sen B.D.S., et al., 2010).

In connection to the above, antioxidants are molecules that can prevent or slow down the oxidation of macromolecules. Antioxidants' role is to reduce or stop this chain reaction by eliminating free radicals or inhibiting other oxidation reactions by oxidizing themselves. Thus, antioxidants are often reducing agents (Always A. et al., 2019). Various antioxidants are currently found in food, namely, natural antioxidants, synthetic antioxidants, dietary antioxidants, and endogenous antioxidants that play an important role in food preservation (Anuj Y et al., 2016). Antioxidants are very important for the health of the body, one of which is anti-aging. The aging process is a natural process that happens from time to time. Both physical and physiological changes can identify the aging process. Physical changes can be characterized by wrinkles, reduced skin elasticity, uneven pigmentation, brown spots,

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weakness, and a rough appearance. It can be caused by free radicals, which can also accelerate the aging process. The formation of ROS can cause oxidative damage and tissue damage in the skin and cause loss of elasticity in the skin (McDaniel D et al., 2018).

Several synthetic antioxidant compounds are available, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertubutylhydroquinone (TBHQ) (Li Z.J et al., 2018). The use of synthetic antioxidants has been reported in several studies to be carcinogenic and may affect organs (Li Z.J et al., 2018) (Chen X et al., 2014). Therefore, as additional antioxidants, the natural antioxidants in diet are more in demand to decrease carcinogenic effects.

Plant based bioactive compounds such as curcumin, quercetin, resveratrol, glucosinolates, Ginger, Biocalein, Genistein, vinca alkaloids (vinblastine, vincristine, vindesine, vinorelbine), taxanes (paclitaxel, docetaxel), podophyllotoxin and its derivations (topothecan, irinothecan), anthracyclines (doxorubicin, daunorubicin, epirubicin, idarubicin), lectines, and xanthorrhizol exerts anticancer effects by several mechanisms (Thanekar et al., 2016).

Phytochemicals like polyphenolic constitute one of the most prominent secondary plant metabolites, which are ubiquitous in the plant kingdom attracted the attention of researchers worldwide as Nutraceutical in the prevention of several diseases. Many studies revealed that anti-cancer efficacy and action of synthetic or natural anticancer compound can be further enhanced by combining them synergistically with chemically similar or different compounds such as poly-phenols which display many anti-carcinogenic properties including their inhibitory effects on cancer cell proliferation, tumour growth, angiogenesis, metastasis, and apoptosis. In addition, they can also modulate immune system.

Many natural antioxidants are found from phenolic compounds such as the flavonoid group. Flavonoids are a group of secondary metabolites produced by plants (Gonzales G.B et al.,

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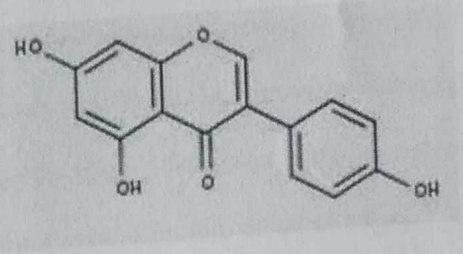
2015). Genistein is one of the most well-known types of isoflavonoids. Genistein [5,7-trihydroxyisoflavone or 5,7-dihydroxy-3- (4-hydroxyphenyl) chromen-4-one] ($C_{15}H_{10}O_5$) belongs to the class of natural isoflavonoid flavonoids with a 15-carbon framework. Isoflavonoids found in beans, especially soybeans (Mazumder MAR and Hongsprabhas P., 2016) (Brodowska K.M., 2017). Genistein is also found in several other plants, which are excellent sources of phytoestrogens such as Lupine (Lupinus spp.), Fava beans (Vicia faba), Psoralea (Psoralea corylifolia), and Kudzu (Pueraria lobata) (Behloul N and Wu G., 2013). The Genistein content of various fruits, nuts, and vegetables can vary widely [Jaiswal N, et al., 2019]. Like other plant constituents, such as Lignans, which have an estrogenic effect, Genistein is a typical example of a phytoestrogen compound. This plant was first isolated from Genista tinctoria L. in 1899 (Spagnuolo C., 2015).



Genistein is a flavonoid that has been discovered naturally in a range of dietary items. Genistein is a flavonoid that was first extracted from the dyer's broom Genista tinctoria in 1899. Prunitol and Genistein are structurally similar. The synthetic chemistry of Genistein was completed in 1928. Genistein has been identified in several types of glycosidic combinations in soy products. Genistein is a phytoestrogen because it has estrogenic actions in the human body. Genistein is structurally identical to 17-estradiol, may disrupt with hormone action through competing processes for receptor site, and has estrogenic impacts; yet, it has non-estrogenic receptor-mediated cellular responses. Genistein also has powerful antioxidant effects. Genistein is an

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important regulator of several signalling pathways at the transcription and translation levels (Tuli H et al. 2019).

Soybeans and soybean derivatives such as soy milk, tofu, soy flour, miso, and natto are high in Genistein. Though, a little amount of Genistein also discovered in various pulses and fruits such as chickpeas, red clover, alfalfa, currants and resins etc. Genistein contains anti-cancer and anti-oxidant properties, and it has been discovered that Genistein promotes glutathione peroxidase activation and limits DNA oxidation in human prostate carcinomas. Genistein is a chemoprotective agent and considered as a treatment therapy for several kinds of cancer including breast tumour, prostate cancer, colorectal cancer, liver tumour, and gastric cancer. Platelet activation and aggregation are critical in the pathophysiology and progression of vascular diseases. Flavonoids derived from plants have a possibility to treat cardiovascular disorders, and Genistein has significant cardioprotective prospective. According to one study, Genistein reduces inflammatory processes and provides cardio-protection in opposition to myocardial ischemia-reperfusion injury. Genistein contains potent biological properties that protect against breast cancer and cardiovascular diseases (Yang Z et al. 2012).

Genistein is one of the most significant phytoestrogens, with amazing biological impacts on the well-being of humans (Rasheed S et al. 2022).

Hence it is also employed for managing effects of menopause such as night sweats, hot flashes, and insomnia, which are frequently caused by a lack of estrogen.

In this piece of work, in vivo studies are intended with regards to the antioxidant potentials of Genistein on human breast cancer cell lines that would be a prelude for studies pertaining to health perspectives of Genistein as an anti-cancer agent.

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2. PURPOSE OF RESEARCH:

- To determine enzymatic and non enzymatic antioxidant activity in cultured human cancer cell lines on Genistein treatment.
- To analyze the mechanism of induction of apoptosis in cancer cells on treatment with Genistein.
- To evaluate the effects of Genistein on oxidant induced damage of DNA in the cultured cell lines.
- To ascertain the potential of Genistein against cancer or itself can act as a good therapeutic agent cancer.

BRIEF REVIEW OF LITERATURE

The flavonoid known as Genistein may be found in a variety of plant-based meals, including fruits and vegetables. It is possible that some of the foods we eat include this molecule, which has qualities that make it effective against inflammation, oxidative damage, bacteria, and viruses. Number of flavonoids is being studied for their possible anticancer effects. Genistein effectively suppressed the growth of triple-negative breast cancer (TNBC) MDA-MB-231 cells. Observations exhibited that Genistein showed inhibitory effects toward cellular growth and was resilient in MDA MB-231 cell lines compared to estrogen receptor-positive BT-474 cell lines. As suggested by published research, Genistein can boost the effectiveness of other therapies or work synergistically with other drugs to produce synergistic effects that are beneficial to the patient. Apoptosis can be caused by Genistein because it causes an increase in the expression of caspase-3, Bax, and p21 while simultaneously causing a decrease in the expression of Akt, cyclin-B1, PLK-1, Cyclin-A, CDK-2, CDC-2, and Bcl-2. In addition to this, it has been established that it raises the total amount of STAT3 protein that is degraded in liver cancer cells

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while simultaneously lowering the total amount of STAT3 activation (Sethi G et al. 2023). Genistein may be particularly effective to induce apoptosis and promote cancer cell death. Researchers like Savoia et al. reported that estrogens and phytoestrogens are promising alternatives to the treatment of the aging process through mechanisms associated with estrogen receptors (ER), guanine nucleotide-binding protein (GPER30) coupled receptors, mitochondrial function, and release of nitric oxide (NO). Research showed that Genistein is a phytoestrogen that can prevent the aging process due to its antioxidant activity. Genistein can decrease the activation/expression of eNOS and iNOS induced by hydrogen peroxide-induced peroxidative stimulation (Savoia P., 2018). In addition to its antioxidant properties and estrogenic/antiestrogenic activity, genistein has several molecular roles, including inhibition of protein tyrosine kinase and Topoisomerase II and degradation of phosphatidylinositol and proteins involved in multidrug resistance in cancer cells. The various cellular roles of Genistein have led to its use as a modulator of cell proliferation, apoptosis, differentiation, and cell cycle development, especially in cancer cells (Jurzak M et al., 2017). Uncontrolled ROS can increase oxidative stress to lead to cell injury and cytotoxicity and may, therefore, destroy DNA, proteins, and lipids (Togliatto G et al., 2017).

In other context, free radicals are constantly produced during oxidative stress, and they are known to be highly toxic and have a deleterious effect on the cellular and enzymatic integrity. Genistein plays an important role on recycling enzymes, which are known as body's main antioxidant constituents. For instance, Genistein induced activation of antioxidant enzymes through AMPK and PTEN pathways in prostate cancer cells (DU145). Genistein also stimulated the expression of the manganese superoxide dismutase and catalase, which collectively led to significant reduction in reactive oxygen species levels (Park et al., 2010). In vivo, genistein exposure governed the non-enzymatic antioxidant action by increasing the expression of

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glutathione and NAD(P)H dehydrogenase (Wiegand et al., 2009; Vanhees et al., 2013). In another study, researchers revealed that Genistein possessed inhibitory action on cancer-associated enzyme, ornithine decarboxylase, and considerably suppressed skin carcinogenesis stimulated by ultraviolet light in a mice model (Record et al., 1995; Mazumder and Hongsprabhas, 2016).

A study by Jin *et al*., 2019, reported that genistein reduced inflammation (Cai C et al,. 2019). NF-κβ act as signals of inflammation and some research suggests that this pathway can enable high lipid, TNF-α, endotoxin, and TLR4 associated with inflammation (Susutlertpanya W et al., 2015). According to Yin *et al*., 2019, it concluded that genistein reduces lipid metabolism and TNF-α and can inhibit the expression of TLR4 and mRNA proteins in inflammatory liver cells by decreasing the level of endotoxins in the blood, thereby preventing synthesis in the liver. Genistein can substantially inhibit ultraviolet (UV) irradiation-induced oxidative processes, including the production of hydrogen peroxide, lipid peroxidation, and 8-hydroxy-2′-deoxyguanosine formation (Taie et al. 2008) (Wei et al. 2002).

4. PROPOSED METHODOLOGY IN BRIEF:

The study would encompass treatment of Genistein to cultured breast cancer cell lines and analysis of various parameters in comparison to untreated control.

A. Antioxidant Analysis

i. Enzymatic antioxidant assay

ii. Non enzymatic antioxidant assay

B. Cytotoxicity Assay

C. Biochemical Assays

D. Statistical Analysis of results would be done using a suitable test of significance.

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5. EXPECTED OUTCOME OF THE PROPOSED WORK:.

The study would help in understanding the antioxidant potential of Genistein which would be a prelude for understanding the mechanisms of interaction of Genistein with cellular molecules like DNA and proteins which are the key factors responsible for regulation of cell cycle and progression of cancer. The study would facilitate in designing the alternative drugs and to decide accurate delivery systems to treat cancer. The present study intends establish Genistein as a candidate drug in the prevention and prognosis of cancer.

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